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PATENT

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IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

U.S. NATIONAL FILING UNDER 35 USC §371

In Re U.S. National Patent Application of:
Conor MULROONEY et al.

From: PCT/GB00/00921 filed March 13, 2000
British Appln. No. 9905580.8 filed March 12, 1999

For: ENZYMATICALLY CATALYSED SIGNAL AMPLIFICATION

PRELIMINARY AMENDMENT

COMMISSIONER FOR PATENTS
Washington, D.C. 20231

ATTN: BOX PCT/WITH FEE

Please amend the above-identified application as follows:

IN THE SPECIFICATION:

Amend the specification by inserting before the first line the sentence: "This Application is a U.S. National filing under §371 of International Application No. PCT/GB00/00921, filed March 13, 2000, claiming priority from British Appln. No. 9905580.8, filed March 12, 1999, now pending (which is hereby incorporated by reference)."

IN THE CLAIMS:

5. A method for detecting a target molecule comprising the steps of:

i) contacting a sample with a locator probe comprising a binding moiety specific for said target molecule and an amplification nucleic acid sequence to produce a target molecule-locator probe complex, said amplification nucleic acid sequence having one or more restriction sites for a restriction endonuclease when hybridised to a complementary strand;

ii) producing an amplification structure bound to any complex produced in the preceding step by performing one or more times the amplification step of treating said sample and locator probe with:

a) a single stranded amplification template comprising:

i) arranged in a 5' to 3' direction:

a) an extension nucleic acid sequence;

b) a hybridisation nucleic acid sequence complementary to the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step and having substantially the same sequence as said extension nucleic acid sequence; and

c) an amplification moiety, being limited in all but the final amplification step to a nucleic acid sequence; and

ii) optionally comprising at least one signal moiety being other than a nucleic acid sequence;

b) a polymerising agent capable of extending the 3' terminus of the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by synthesising a complementary strand to said extension nucleic acid sequence of said amplification template;

c) said restriction endonuclease; and

d) the reagents and conditions necessary to:

i) effect the action of said polymerising agent and separating agent to allow the extension of the 3' terminus of the amplification nucleic acid sequence of the previous amplification step or, where there is no previous amplification step, of the preceding step by the synthesis of a plurality of sequences complementary to said extension nucleic acid sequence of said amplification template; and

ii) effect dissociation of fragments of nucleic acid strands which have been cut by said restriction endonuclease activity from uncut complementary strands whilst not effecting dissociation of uncut nucleic acid strands from uncut complementary strands;

iii) detecting any bound amplification template from the amplification step or steps; and

iv) correlating the results of detection step (iii) with the presence of said target molecule.

8. A method according to claim 5, being performed isothermally.

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9. A method according to claim 5, being performed at more than one temperature.

10. A method according to claim 1, the amplification step of step (ii) being performed two or more times.

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13. A method for detecting a target molecule according to claim 11, the removal of said amplification template being achieved by the use of a 5' double strand specific exonuclease.

14. A method for detecting a target molecule according to claim 11, the removal of said amplification template being achieved through the use of elevated temperature.

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16. A method for detecting a target molecule according to claim 1, prior to said detection step additionally comprising performing a method according to steps (ii) and (iii) of claim 11.

17. A method for detecting a target molecule according to claim 1, said amplification moiety of said amplification template from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing steps (ii)-(iii) of a method according to claim 11.

18. A method for detecting a target molecule according to claim 11, prior to said detection step additionally comprising performing step (ii) of a method according to claim 1.

19. A method for detecting a target molecule according to claim 11, said amplification moiety of said locator probe or additional locator probe from said final amplification step comprising a nucleic acid sequence, and prior to said detection step additionally comprising performing step (ii) of a method according to claim 1.

20. A method for detecting a target molecule according to claim 1, the step of detecting any bound amplification template comprising the steps of:

i) treating said sample, locator probe and amplification template or amplification templates with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and

ii) detecting any bound detection probe.

21. A method for detecting a target molecule according to claim 12, the step of detecting any bound amplification template comprising the steps of:

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i) treating said sample, locator probe and amplification template with a detection probe which binds specifically to said amplification moiety of the last of said amplification templates; and

ii) detecting any bound detection probe.

22. A method according to claim 20, the detection probe having a label which is detected by any one of the group of luminometry, fluorometry, spectrophotometry, and radiometry.

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24. A method according to claim 1, the amplification step being performed two or more times, each amplification step being performed using an amplification template having a different extension nucleic acid sequence, hybridisation nucleic acid sequence and amplification moiety to that of the amplification template used in the previous amplification step.

25. A method according to claim 1, the target molecule to be detected being a nucleic acid sequence and the binding moiety of said locator probe comprising a nucleic acid sequence complementary to said target molecule nucleic acid sequence.

26. A method according to claim 1, being performed using more than one locator probe, each locator probe having the same amplification nucleic acid sequence.

27. A method according to claim 1, comprising two repeats.

28. A method according to claim 1, unreacted reagents being removed at the end of step (i), each repeat, or detection step by washing.

Please add the following new claims:

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30. A method according to claim 3, the amplification step of step (ii) being performed two or more times.

31. A method according to claim 5, the amplification step of step (ii) being performed two or more times.

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32. A method for detecting a target molecule according to claim 12, the removal of said amplification template being achieved by the use of a 5' double strand specific exonuclease.

33. A method for detecting a target molecule according to claim 12, the removal of said amplification template being achieved through the use of elevated temperature.

34. A method according to claim 21, the detection probe having a label which is detected by any one of the group of luminometry, fluorometry, spectrophotometry, and radiometry.

35. A method according to claim 34, the detection probe being labelled with any one of the group of, FAM (carboxyfluorescein), HEX (hexachlorofluorescein), TET (tetrachlorofluorescein), ROX (carboxy-X-rhodamine), TAMRA (carboxytetramethylrhodamine), JOE (carboxy-4',5'-dichloro-2',7'-dimethoxyfluorescein), or with biotin.

These amendments are being submitted in the form required under the amended rule 37 CFR 1.121. Accordingly, sheets containing the marked-up claims corresponding to the substitute claims below are attached.

No new matter is being added through these amendments. Applicant respectfully requested entry of the above amendments.

Respectfully submitted,

Date: September 10, 2001 By: Monique A. Morneault

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